BARD Final Report IS-4576-13 R

**A novel approach for preventing *Myxozoan* infection in fish: Control of polar capsule activation**

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Project award year: 2013
Three year research project

## Abstract

### List of the original objectives:

1. Isolate polar capsules and test them to reveal their role in adhesion and/or as injectors.
2. Analyze the polar capsule protein content.
3. Characterize the capsule envelope properties (charge, adsorption, etc.) and determine the role of the permeability coefficient on controlling water and solute fluxes during filament release and any peptide injection.
4. Investigate the effects of osmotic pressure, viscosity and filament geometry (etc.) on capsule discharge and mass flow rate in the filament.
5. Quantify the fast filament release and injection dynamics using a microfluidic platform.
6. Develop experimental and numerical analysis scheme to model the capsule system.
7. Develop and test potential therapeutic agents in fish infection challenges.

### Background to the topic

Myxozoans have a worldwide distribution and are an important group of microscopic parasites that affect many freshwater and marine fish hosts. They have complex life cycles with two spore stages that develop alternately in vertebrate (mostly fish) and invertebrate (mostly worm) hosts. They present a major threat to fish hatcheries and aquaculture by affecting growth and causing mortality through diseases that include proliferative kidney disease, ceratomyxosis, and whirling disease. Yet, despite their devastating impact on commercial aquaculture, no effective therapeutants are known. The first step in the myxozoan infection process is activation of their polar capsules, specialized organelles akin to nematocysts, the stinging cells of free-living cnidarians. Polar capsules contain coiled and eversible polar tubules that are fired out and anchor the spore to the host, thereby facilitating penetration and infection. The objective of the proposed research was to better characterize polar capsule activation processes by using a multidisciplinary approach that combines biology, fish parasitology, microfluidics and numerical modeling.

### Major conclusions, solutions, achievements

1. Polar capsules were isolated for the first time using especially designed dielectrophoresis (DEP) based filtering microfluidic chip.
2. Polar capsules from different species behave differently. *Ceratomyxa shasta* tubules have no opening, suggesting a role in anchoring. Conversely, *Myxobolus* polar capsules can inject their contents to their host, suggesting an active role during the infection.
3. The proteomic content *of C. shasta* polar capsules demonstrated high similarity to cnidarian nematocysts, suggesting similar mechanism of activation; however, no toxins were identified.
4. Using microfluidics and mathematical modeling, we showed that the driving force of tubule elongation is an osmotic potential developing inside the tubule with its highest value at the tubule leading edge and that the internal capsule pressure is not sufficient to drive complete tubule release.
5. High-speed video analysis of tubule discharge of three *Myxobolus* species revealed kinetics and elasticity that are different from the known free-living cnidarians.
6. Fish infection tests using potential polar capsule inhibiting compounds demonstrated the ability to prevent infection.

### Implications, both scientific and agricultural

The characterization of the capsule content, discharge and tubule elongation mechanism provides new insights into the evolution of Myxozoa from their free-living ancestors and paves the way for the development of new tools for parasite management in aquaculture.

## Summary Sheet

## Publication Summary

| PubType | IS only | Joint | US only |
|---|---|---|---|
| Reviewed | 2 | 2 | 1 |
| Submitted | 1 | 0 | 0 |

## Training Summary

| Trainee Type | Last Name | First Name | Institution | Country |
|---|---|---|---|---|
| Postdoctoral Fellow | Lovy | Alena | University of Haifa | Israel |
| Ph.D. Student | Ban David | Jonathan | University of Haifa | Israel |
| M.Sc. Student | Piriatinskiy | Gadi | University of Haifa | Israel |
| M.Sc. Student | Zeevi | Dan | Technion | Israel |
| Postdoctoral Fellow | Park | Sinwook | Technion | Israel |
| Ph.D. Student | Rosentsvit | Leon | Technion | Israel |
| M.Sc. Student | Murphy | Laura Taggart | Oregon State University | USA |
| Ph.D. Student | Barrett | Damian | Oregon State University | USA |
| M.Sc. Student | Lawrence ( undergraduate) | Emily | Oregon State University | USA |
| M.Sc. Student | Nawsher (undergraduate) | Molla Meher | Oregon State University | USA |
| Postdoctoral Fellow | Huo | Xiaoye | Technion | Israel |
| M.Sc. Student | Bahar (undergraduate) | Eyal | Technion | Israel |

**Details of cooperation**

The four groups have maintained regular contact via email and video conferences. Information shared between the groups includes newly obtained genomic and transcriptomics data of the tested spores. The proteomic analysis (Israel) was based on transcriptomic drafts of the US group. There have been constant shipments of myxozoan spores from US to Israel. Spores were purified in US and then shipped to Israel for isolation of the polar capsules (Lotan and Yossifon). The high-speed analysis and analysis of the polar capsule injection kinetics (Lotan, Shavit and Yossifon) were done on spores isolated in the US. Deciphering the tubule release mechanism (Lotan, Shavit and Yossifon) contributes to our understanding of the polar capsule firing (US and Israel).

A face-to-face meeting of Lotan, Bartholomew, Atkinson and Ben David took place in August-September 2015 at the International Symposium of Fish Parasites in Valencia, Spain, where we also presented our findings through an oral talk and posters. Ben David's poster won the Best Poster Presentation Award (from over 200 contenders). Additionally, in September 2016 Lotan visited Bartholomew and Atkinson at OSU to finalize our findings.

**Achievements**

**Significance of main scientific achievements or innovations**

The project represents the first targeted investigation of the initial steps of the myxozoan infection process, using combined physical, chemical, mathematical and biological analyses. In addition, two novel infectious *Myxobolus* species were characterized in the US and in Israel [1, 6].

*Polar tubule structure:* SEM analysis of *Myxobolus* polar capsule tubules revealed their unique ultrastructure of double spirals of nodules and pores along the tubule, and showed that the distal tip of the tubules was sealed. This helical pattern and distribution of openings are likely needed to improve the mechanical resistance required by the tubule to cope with the stresses generated during the firing stage [1, 2].

*Polar tubule kinetics and injection capability:* High-speed video analysis of the firing process from the three *Myxobolus* species showed that all polar tubules rapidly extended and then contracted, an elasticity phenomenon that is unknown in free-living cnidarians. Interestingly, the duration of the tubule release differed among the three species by more than two orders of magnitude, ranging from 0.35 to 10 s, and only two of the three species could inject their content [1, 2].

*Isolation and characterization of polar capsules:* We developed a tailored dielectrophoresis-based microfluidic chip for isolation of *C. shasta* myxospore polar capsules [3]. We have also shown in nematocysts that the DEP method allows further characterization of the ionic content of the capsules [4]. Using electron microscopy and functional analysis, we demonstrated that *C. shasta* tubules have no openings and are likely used to anchor the spore to the host [3]. Proteomic analysis of *C. shasta* polar capsules suggested that they have retained typical structural and housekeeping proteins found in nematocysts of jellyfish, sea anemones and *Hydra*, but have lost the most important functional group in nematocysts, namely the toxins. The osmotic machinery that drives tubule ejection in free-living cnidarians is based on a unique anionic matrix of pγGlu. Our finding of pγGlu-related enzymes in *C. shasta* capsules together with intense cationic dye staining of the capsule content may indicate that the same principal function of tubule firing is shared between capsules of parasitic and free-living species [3]. Our results support the hypothesis that polar capsules and nematocysts are homologous organelles that have adapted to their distinct functions.

*Nematocyst tubule elongation:* Based on our finding that polar capsules and nematocysts display a similar mechanism of discharge, we have used jellyfish nematocysts, which are much easier to

obtain, as a model system for understanding how the build-up of pgGlu-based osmotic potential inside the capsule drives its discharge. To control the osmotic potential, we used a microfluidic system consisting of two opposite wide microchannels, connected by an array of narrow microchannels (~5μm in width), to direct the elongating nematocyst tubule through oil, where no osmotic potential can develop, while keeping the capsule in water at all times [5]. Flow inside the tubule and the pγGlu concentration profiles were calculated by applying a one-dimensional mathematical model. We found that tubule elongation through oil is orders of magnitude slower than through water and that the injection rate of the capsule content is reduced. These results imply that the capsule's osmotic potential is not sufficient to drive the tubule beyond the initial stage. Our proposed model shows that the tubule is pulled by the high osmotic potential that develops at the tubule moving front [5].

*Fish infection challenges test:* We tested a range of salts (chlorides of Ca, K, Mg, Fe and Gd-a Ca channel blocker) and diphenhydramine (a known cnidarian cnidocyte receptor disruptor) to determine their efficacy at inhibiting the infection process. Individual rainbow trout (*O. mykiss; N* = 3 each salt/concertation) were exposed to 500 – 1,000 actinospores from polychaete worm cultures in the presence of the test substances. We tested the effect of salts at 1-100mM concentrations except for Gd and diphenhydramine, which were tested at micromolar levels (already known to affect free living Cnidarians). Fish could not tolerate Fe at any concentration above 1mM or diphenhydramine above 50uM. Fish tolerated all other salts at the maximum concentration of 100mM and this salt concentration prevented infection (likely due to osmotic disruption of actinospores). It was also found that iron blocked infection at all tested levels. Diphenhdramine blocked infection at 50um or above, whereas Gd only partly blocked the infection at the highest tested concentration (500uM). Calcium performed like a positive control, only blocking the infection at the highest concentration (100mM).

**Agricultural and/or economic impacts of the research findings, if known**.

Although a natural part of most aquatic ecosystems, Worldwide, myxozoans have significant effects on the growth, marketability and survival of fisheries, particularly those where fish are mass cultured. Treatment options for myxozoan disease have remained elusive. Results of this study demonstrate fundamental structural and functional differences between free-living and parasitic Cnidaria, and define new and promising targets for further research. We found that certain

compounds can block the myxozoan infection process and may represent avenues for design of treatments against myxozoan infection in commercial settings.

**List of Publications:**

1 Atkinson SD, Banner CR: A novel myxosporean parasite *Myxobolus klamathellus* n. sp. (Cnidaria: Myxosporea) from native blue chub (Gila coerulea) in Klamath Lake, Oregon. Parasitology Research 2017: 116:299–302.

2. Ben-David J, Atkinson SD, Pollak Y, Yossifon G, Shavit U, Bartholomew JL, Lotan T: Myxozoan polar tubules display structural and functional variation. Parasites & Vectors 2016;9:549.

3 Piriatinskiy G., Atkinson SD, Park S, Morgenstern D, Brekhman V, Yossifon G, Bartholomew JL, Lotan T: Functional and proteomic analysis of *Ceratonova shasta* (Cnidaria: Myxozoa) polar capsules reveals adaptations to parasitism. Scientific Reports 2017;7:9010.

4 Park S, Capelin D, Piriatinskiy G, Lotan T, Yossifon G: Dielectrophoretic characterization and isolation of jellyfish stinging capsules. Electrophoresis 2017; 38:1996 (we got the front cover of the journal).

5 Park S, Piriatinskiy G, Zeevi D, Ben-David J, Yossifon G, Shavit U, Lotan T: The nematocyst's sting is driven by the tubule moving front. Journal of The Royal Society Interface 2017;14:20160917.

6 Lövy A, Smirnov M, Brekhman V, Ofek T, Lotan T: Morphological and molecular characterization of a novel myxosporean parasite *Myxobolus bejeranoi* n. sp. (Cnidaria: Myxosporea) from hybrid tilapia in Israel, Under review.

## Publications for Project IS-4576-13 R

| Status | Type | Authors | Title | Journal | Vol:pg Year | Coun |
|---|---|---|---|---|---|---|
| Published | Reviewed | *Ben-David J, Atkinson SD, Pollak Y, Yossifon G, Shavit U, Bartholomew JL, Lotan T* | Myxozoan polar tubules display structural and functional variation | ***Parasites & Vectors*** | 9 : 549 2016 | Joint |
| Published | Reviewed | *Piriatinskiy G., Atkinson SD, Park S, Morgenstern D, Brekhman V, Yossifon G, Bartholomew JL, Lotan T* | Functional and proteomic analysis of Ceratonova shasta (Cnidaria: Myxozoa) polar capsules reveals adaptations to parasitism | ***Scientific Reports*** | 7 : 9010 2017 | Joint |
| Published | Reviewed | *Park S, Capelin D, Piriatinskiy G, Lotan T, Yossifon G* | Dielectrophoretic characterization and isolation of jellyfish stinging capsules | ***Electrophoresis*** | 38 : 1996-2003 2017 | IS only |
| Published | Reviewed | *Park S, Piriatinskiy G, Zeevi D, Ben-David J, Yossifon G, Shavit U, Lotan T* | The nematocyst's sting is driven by the tubule moving front | ***Journal of The Royal Society Interface*** | 14 : 20160917 2017 | IS only |
| Published | Reviewed | *Atkinson SD, Banner CR* | A novel myxosporean parasite Myxobolus klamathellus n. sp. (Cnidaria: Myxosporea) from native blue chub (Gila coerulea) in Klamath Lake, Oregon | ***Parasitology Research*** | 116 : 299-302 2017 | US only |
| Submitted | Reviewed | *Lovy A, Smirnov M, Brekhman V, Ofek T, Lotan T* | Morphological and molecular characterization of a novel myxosporean parasite Myxobolus bejeranoi n. sp. (Cnidaria: Myxosporea) from hybrid tilapia in Israel | | : | IS only |